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A CONTRIBUTION TO THE LIFE HISTORY OF ASTER.

MARIE OPPERMAN.

(WITH PLATES XIV AND XV)

MATERIALS AND METHODS.

THIS study is based upon an investigation of *Aster undulatus*, *A. multiflorus*, *A. Novi-Belgii*, and *A. Novae-Angliae*. Flowers in various stages of development were collected. The buds were halved, the smaller heads were cut into thirds, and the larger into quarters. The upper portion of the floral envelopes together with the inclosed styles and stamens were cut away, great care being exercised not to injure the ovules. Two fixing fluids were used, Flemming's chrom-osmo-acetic acid mixture and chrom-acetic acid, both giving good results. After dehydration the material was brought gradually, through ascending grades, into either cedar oil or chloroform before infiltration with the paraffin. So far as a study of my material goes, it is a mere matter of convenience whether cedar oil or chloroform be used in the process of imbedding, as the results obtained by either method were equally satisfactory. Sections were cut 10 μ thick and stained in Flemming's safranin, gentian-violet, and orange combination, or in Heidenhain's iron-haematoxylin. When Flemming's triple stain was used, it was necessary to allow the slides to remain for several hours in the gentian-violet, since this stain is rapidly removed during the process of dehydration in Aster. It was found advantageous to place the slides in xylol before mounting in balsam. With this treatment the sections cleared more quickly, and were therefore ready for study in a shorter time than when mounted directly from the bergamot oil.

This investigation was carried on in the Botanical Laboratory of Wellesley College, under the direction of Dr. Margaret C. Ferguson, to whom I desire to express my gratitude for her invaluable counsel and unceasing interest and encouragement.

THE DEVELOPMENT OF THE EMBRYO SAC.

The embryo sac arises from the lowest cell of an axial row of four cells, that is, from the cell most distant from the micropyle. The mother-cell of the embryo sac grows rapidly, soon becoming strongly differentiated from the surrounding cells. At some time during the early stages of development, the nucleus of the macrospore moves to the micropylar end of the spore and there divides. The embryo sac contains very little cytoplasm at this stage, with the exception of the dense mass around its nucleus (*fig. 1*). It agrees in this respect with the young embryo sac of Silphium, as described by Merrell (1900), and differs from Lilium and some other plants in which the embryo sac is densely filled with cytoplasm at the time of the first nuclear division.

In *A. multiflorus* the young embryo sac reaches an advanced stage of growth at the time of the first division of its nucleus, and does not show a very great increase in size subsequent to its binucleate stage. In the other species studied, however, the embryo sac does not assume its ultimate form until about the time of the fusion of the polar nuclei. After the division of the macrospore nucleus, one of the resulting nuclei moves toward the opposite or lower pole. In the meantime the embryo sac continues to grow in length, but increases very little, if any, in breadth. At the time of the second nuclear division, the embryo sac is long and somewhat cylindrical in outline. Here, as in other angiosperms, the two nuclei divide simultaneously, the division occurring, in some cases at least, before the lower nucleus has reached the antipodal end of the sac (*figs. 2, 3*). In the embryo sac shown in *fig. 2* the second nuclear division is just completed, and the nuclei have not as yet separated. The four nuclei enter the complete resting stage, as shown in *fig. 3*, and then immediately divide again, giving rise to a typical eight-nucleate embryo sac.

At the time when the eight nuclei are formed, the embryo sac, as seen in vertical section, is more or less rectangular in outline, but from this stage on the two poles of the embryo sac may become strongly differentiated. The antipodal end shows little or no increase in breadth, but elongates rapidly, pushing down for a considerable distance into the nucellus, while the upper and central region of the embryo sac ceases to elongate, but grows to several times its original breadth.

Considerable variation has been noted in the shape and size of the mature embryo sac, not only in the different species, but also in different ovules of the same species. These irregularities may be apparent at an early stage, as is shown in *figs. 2* and *3*. The embryo sac represented in *fig. 3*, although of the same species as the one represented in *fig. 2* and of a later stage of development, has grown much less in length. It is also apparent from a comparison of *figs. 6* and *7* that, while the embryo sac of *A. undulatus* shows a remarkable growth in breadth near the micropylar end, the embryo sac of *A. multiflorus*, though slightly older than the former, has broadened very little in that region. The upper portion of the embryo sac of *A. Novi-Belgii*, like that of *A. multiflorus*, grows little in breadth, while the form of the embryo sac in *A. Novae-Angliae* (*fig. 8*) resembles very much that of *A. undulatus*.

Soon after the embryo sac has reached the eight-nucleate stage of development, three of the nuclei in the lower end of the embryo sac move downward to form the cells of the elongated antipodal region. The four nuclei in the micropylar end assume their characteristic arrangement and give rise to the egg apparatus and to the upper polar nucleus. The egg nucleus rests in the lower oval end of a pear-shaped cell, in a dense mass of cytoplasm. Above the nucleus the usual vacuole is found, which is very large in Aster, often occupying two-thirds of the entire oosphere. The synergids lie one on each side or both on the same side of the oosphere. Each may have the same relative position with reference to the micropyle, or one synergid may extend farther into the micropyle than the other. They are usually densely filled with cytoplasm, but may be somewhat vacuolate. The nucleus of each synergid is much smaller than the egg nucleus or the upper polar nucleus, and may be placed centrally or at a point considerably above or below the middle of its cell.

Immediately after the organization of the egg apparatus, the polar nuclei move toward one another. The lower nucleus travels much faster than the upper one, so that they meet comparatively near the oosphere. The two take a position just beneath the egg, but in contact with it, and there fuse to form the endosperm nucleus. An early stage of fusion is illustrated by *fig. 6*, in which one polar nucleus appears closely pressed against the other. In *fig. 7* the membranes between the two fusing nuclei have already disappeared. The endosperm nucleus is very large, almost spherical in outline, and is furnished with a rather open reticulum and one large densely staining nucleolus. The fusion of the polar nuclei may be delayed, in some instances until the time of fertilization (*fig. 9*), but ordinarily the formation of the endosperm nucleus has taken place before the pollen tube discharges its contents into the embryo sac.

When the embryo sac is mature, it occupies about one-half the entire length of the nucellus. The lower two-thirds of the embryo sac consists of the narrow elongated antipodal cells. As a rule, the cells of the nucellus immediately surrounding the antipodals are empty and more or less irregular in outline. A general conception of the structure and position of the parts of the ovule at the time of the maturity of the embryo sac is given in *fig. 10*.

LATER HISTORY OF THE ANTIPODALS.

The antipodal portion of the embryo sac in dicotyledons has been found to vary greatly in the number, structure, and position of its cells. Chamberlain (1895) finds that the number of antipodals in *A. Novae-Angliae* ranges from two to thirteen, six or seven being the usual number. The lower antipodal was found to differ from the others in size, in the density of its cytoplasm, and in the appearance of its nuclei. He observes, however, that in one instance the contents of this cell bear a superficial resemblance to an egg and believes that it represents an antipodal oosphere. The number of nuclei in an antipodal cell was found to vary from one to over twenty. In my study of Aster I find that the antipodals in the species examined, although showing in no instance as many as thirteen cells, agree closely, as to their structure, with Chamberlain's description, but they do not confirm the observations made by Martin (1892), who found only four antipodals. These four cells were never arranged in a single longitudinal row, and he did not figure them as separated by cell walls.

Definite cells appear in the antipodal region very early in the development of the embryo sac. As a rule, these cells have reached their greatest development when the embryo sac is ready for fertilization, but occasionally they continue to grow for some time after fertilization. It often occurs in *A. Novae-Angliae* and in *A. undulatus* that the lowest of the antipodal cells becomes very much enlarged (*figs. 11, 14, 16*). The nuclei of this cell were found in a few cases to be somewhat larger than the nuclei of the other antipodals, but in no instance was I able to find in this lowest cell an antipodal oosphere as described by Chamberlain (1895). However, an egg, doubtless derived from one of the upper antipodal nuclei, was observed and will be described later. In one preparation an embryo sac was found in which the lowest antipodal exceeded in width the expanded upper portion of the embryo sac, but there was nothing in the character of its nuclei to indicate the presence of an antipodal egg (*fig. 16*).

There seems to be no limit to the number of antipodal nuclei in Aster. Cells which contained only one nucleus were found in only a few cases. *Figs. 14* and *15* illustrate two antipodal cells, one of which contains fourteen nuclei of various sizes and the other nineteen small nuclei.

The antipodals persist until the embryo sac is in an advanced stage of development, and their function is probably that of conveying nutrition to the developing embryo. In several instances two or three cells just below the antipodals were observed to retain their normal appearance, containing cytoplasm and nucleus (*fig. 5*). It may be that these cells are active in supplying food to the developing embryo, as described by Ferguson (1901) in Pinus and confirmed by Coker (1902) in Taxodium.

FERTILIZATION.

Double fertilization has been found to occur in several Compositae and, judging from some of my preparations, it occurs also in Aster; but my material has yielded so few stages just before and including the fusion of the sexual nuclei that I do not wish to speak unqualifiedly regarding this point at present. In *fig. 9* we have an instance of the delayed fusion of the polar nuclei, and apparently an example of double fertilization in *A. undulatus*. The polar nuclei are in an early stage of fusion, and the sperm nucleus is pressed against the lower polar nucleus. The fusion of the sperm with the egg nucleus is nearly completed, so that the two appear almost as one nucleus with two nucleoli.

A most interesting embryo sac is illustrated in *fig. 18*, *a–b*. There can be no doubt that we have in this instance the occurrence of two oospheres and two endosperm nuclei in the same embryo sac. The upper part of the embryo sac resembles all normal embryo sacs at a similar stage of development; but in the lower portion, just above the first antipodal cell, there is a cell and a large nucleus which have the same structure and the same relative position as that of the egg and the endosperm nucleus at the micropylar end. These clearly represent, so far as position is concerned, an antipodal oosphere and a definitive nucleus.

No positive statement regarding the origin of this extra egg and endosperm nucleus can be made, but several theories as to their probable derivation may be suggested. It is possible that the lower polar nucleus did not fuse with the upper polar nucleus, but that each has taken on the appearance of an endosperm nucleus, and that the lower oospere has arisen directly from an antipodal; it may be that the normal egg has wandered to the antipodal region of the embryo sac, and that the upper egg has been derived from one of the synergids; or it is possible that the lower egg has developed from one of the synergids which had become displaced. In order to explain satisfactorily the structure of this embryo sac on the basis that one of the eggs has been derived from a synergid, it is necessary to assume that at least three irregularities have occurred in its development.

Moreover, the two small nuclei at the micropylar end of the embryo sac doubtless represent the synergids, now in a state of disintegration. Therefore, it seems to the writer most improbable that either egg had its origin in a synergid. I am aware that one of the two nuclei just above and at one side of the upper egg may represent the tube nucleus, but we have no direct evidence that such is the case, and it has not yet been demonstrated that the tube nucleus enters the embryo sac in Aster. Judging from the structure of the micropylar end of this embryo sac and from the position of the unusual nuclei, the most probable, and also the most simple interpretation of this phenomenon is that the upper egg apparatus was formed in the usual way, and that the lower egg and the endosperm nucleus associated with it were derived from one of the three original antipodal cells.

We have here not only the remarkable occurrence of a distinctly outlined egg associated with a large definitive nucleus in the antipodal region of the embryo sac, but also the interesting phenomenon of the fertilization of that egg. Whether a sperm nucleus has already fused with the upper egg or with the endosperm nucleus cannot be ascertained. One of the sperm nuclei, however, has passed to the lower part of the embryo sac to fuse with the lower oosphere. The sperm is more or less banana-shaped and is just pressing itself against the cytoplasm of the egg. It has somewhat the appearance of the sperm nuclei of *Paris quadrifolia*, as described in the recent paper of Ernst (1902).

This discovery of an antipodal functioning as an egg and about to be fertilized is a very significant fact, and has an important bearing on the problem of the homologies of the antipodals. It has been shown by different investigators that all the nuclei of the embryo sac except the antipodals are capable of being fertilized and may therefore be considered as potential eggs. As already stated, Chamberlain (1895) reports the occurrence of a nucleus in the enlarged end cell of the antipodals, which he believes to be an oosphere, formed by one of the nuclei of the antipodal region surrounding itself with cytoplasm, and he suggests that we need only to observe an actual case of fertilization in this region to demonstrate the egg-like nature of these cells. Tretjakow (1895) reports an instance in which he finds an embryo in the antipodal region, but as he did not find fertilization he believed the embryo to have arisen apogamously.

Of the two views held as to the homologies of the antipodals, namely, that they represent either a vegetative prothallus or that they are potential eggs, the discovery of the fertilization of an antipodal egg adds substantial evidence to the theory that all the cells of the embryo sac are potential eggs. If the antipodals are potential eggs, then it would seem that the fusion of one sperm nucleus with the two polar nuclei is, as Nawaschin and some other investigators believe, a true act of fertilization.

After fertilization the egg nucleus becomes spherical but does not divide until sometime subsequent to the first division of the endosperm nucleus. The first division of the egg is transverse, as usual, and the subsequent development of the embryo differs little from that of other Compositae.

SUMMARY.

The embryo sac arises from the lowest cell of an axial row of four cells.

The eight-nucleate embryo sac is formed in the usual manner.

Great variation is shown in the form of embryo sacs, both in different species and in the same species.

The polar nuclei fuse ordinarily before fertilization, but may fuse after it.

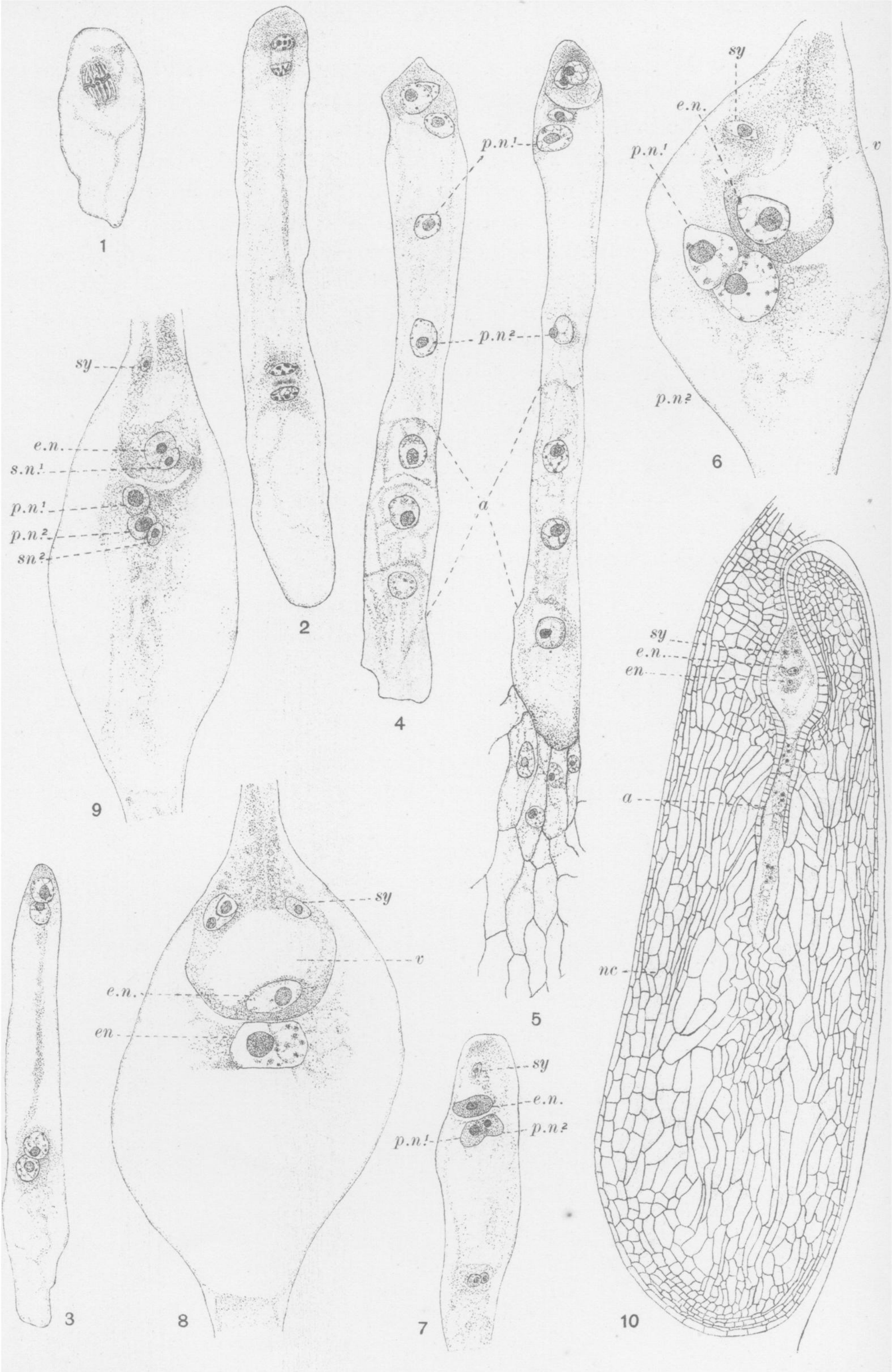
The endosperm nucleus is marked by its very large size, its almost spherical shape, its large nucleolus, and its position below and in contact with the oosphere.

The antipodals show a remarkable growth before the time of fertilization and persist very late in the development of the embryo.

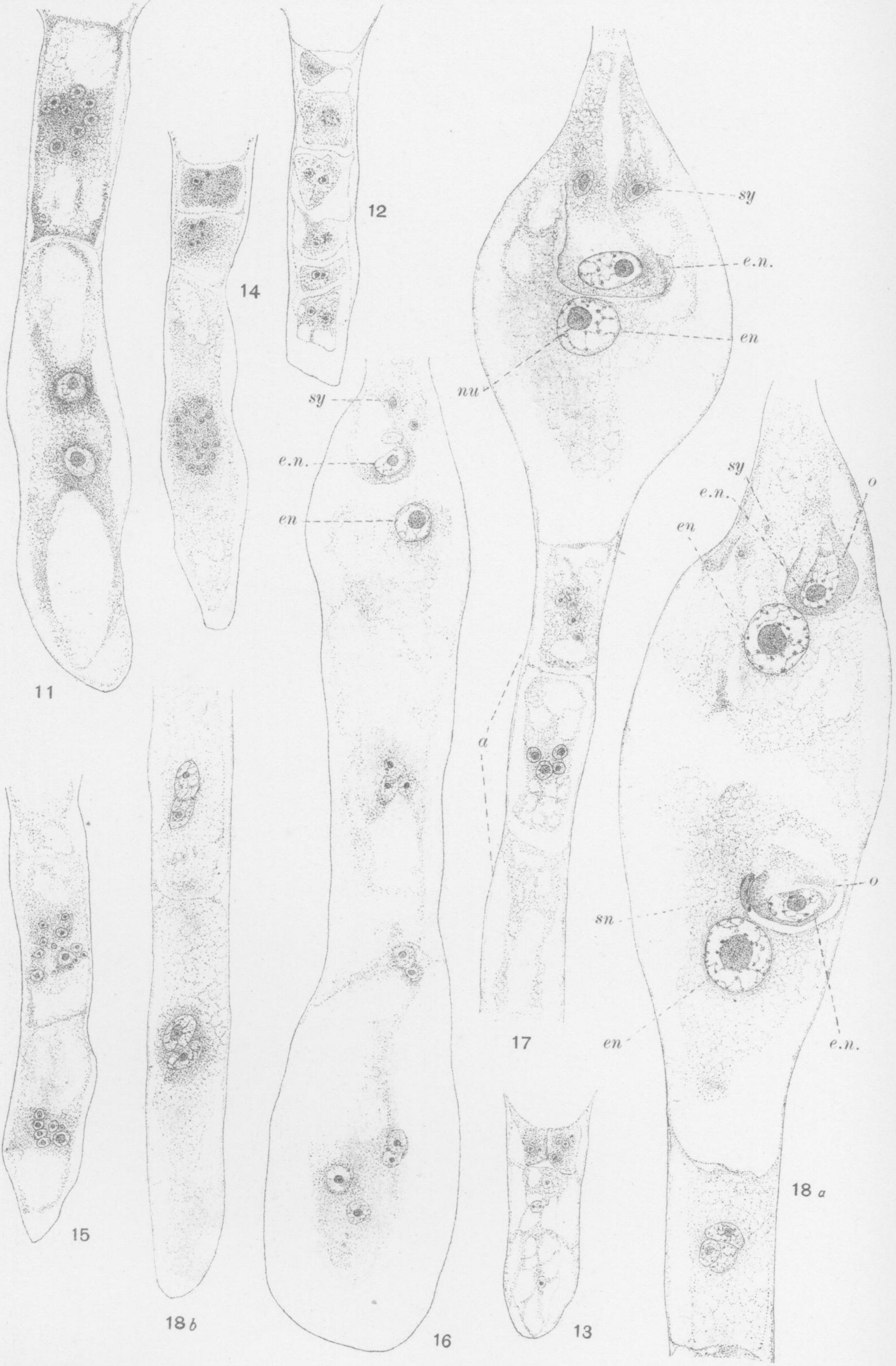
From one to nineteen nuclei were found in a single antipodal cell.

The lowest antipodal cell in *A. undulatus* and *A. Novae-Angliae* is often very much enlarged.

The fertilization of an antipodal egg is clearly demonstrated in Aster.



OPPERMAN on ASTER.



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Double fertilization was observed in *A. undulatus*.

The sperm nuclei assume the structure of ordinary nuclei at the time of their fusion with the egg and the endosperm nucleus.

The development of the embryo follows the type described for other Compositae.

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EXPLANATION OF PLATES *XIV AND XV*.

All figures were drawn with a Nachet camera lucida and are reduced one-fourth in reproduction. The portion of a figure nearest to the micropylar end of the ovule is always toward the top of the plate. The lettering of the figures is to be interpreted as follows: *e.n.* egg nucleus; *o*, oosphere; *sy*, synergids; $p.n.^1$ upper polar nucleus; $p.n.^2$ lower polar nucleus; *nu*, nucleolus; *en*, endosperm nucleus; $s.n.^1$, $s.n.^2$ sperm nuclei; *a*, antipodals; *nc*, nucellus; *v*, vacuole.

FIG. 1. *A. multiflorus*. A young embryo sac with its nucleus in the metaphase of division. × 600.

FIG. 2. *A. undulatus*. A young embryo sac in the two-nucleate stage; the nuclei are in the telophase of division. × 600.

FIG. 3. *A. undulatus*. A four-nucleate embryo sac just after the second nuclear division. × 600.

FIG. 4. *A. Novae-Angliae*. An eight-nucleate embryo sac; the three antipodal cells have just been laid down; the egg apparatus is being formed

in the micropylar end; the polar nuclei are moving toward one another × 600.

FIG. 5. *A. Novae-Angliae.* An eight-nucleate embryo sac; the egg apparatus is not yet formed; the polar nuclei are some distance apart; three active nuclei lie in the nucellus below the antipodal region. × 600.

FIG. 6. *A. undulatus.* The polar nuclei in the first stage of fusion. × 600.

FIG. 7. *A. multiflorus.* The polar nuclei in a more advanced stage of fusion. × 600.

FIG. 8. *A. Novae-Angliae.* A mature embryo sac. × 600.

FIG. 9. *A. undulatus.* An embryo sac in which the fusion of the polar nuclei has been delayed and double fertilization is taking place. × 600.

FIG. 10. *A. undulatus.* A mature embryo sac showing the structure of the surrounding nucellar tissue. × 140.

FIG. 11. *A. Novae-Angliae.* The antipodal region of a mature embryo sac, showing a long somewhat enlarged lower cell with two large nuclei. × 600.

FIG. 12. *A. multiflorus.* The antipodal region of a young embryo sac, showing six cells. × 600.

FIG. 13. *A. multiflorus.* The antipodal region of a young embryo sac, showing two of its cells lying side by side. × 600.

FIG. 14. *A. undulatus.* The antipodal region of an older embryo sac; the lowest cell is enlarged and contains nineteen small nuclei. × 600.

FIG. 15. *A. Novae-Angliae.* The antipodal region of a mature embryo sac showing multinucleate cells. × 600.

FIG. 16. *A. undulatus.* An embryo sac with an enlarged antipodal cell. × 600.

FIG. 17. *A. undulatus.* A normal mature embryo sac. × 600.

FIG. 18, *a. A. undulatus.* An abnormal mature embryo sac, containing an antipodal endosperm nucleus and also an antipodal oosphere which is about to be fertilized by a banana-shaped sperm. × 600.

FIG. 18, *b.* The antipodal cells of the embryo sac in *fig. 18, a.*